P-21: Biophysics

C. C. Wood, Group Leader

Introduction

The Biophysics Group (P-21) was founded in 1988 with the goal of applying the scientific and technical resources of Physics Division to the biosciences. Our mission is to contribute to an understanding of biological phenomena by means of the scientific, technical, and conceptual resources of physics; to use biological systems to elucidate general physical principles underlying complex phenomena; and to apply, where appropriate, our scientific and technical capabilities to core Laboratory programs.

Just as the 20th century is regarded as the century of the physical sciences, the 21st century will likely become the century of the biological sciences. P-21 and biophysics as a discipline are well-positioned to contribute to this biological revolution-in-progress through our emphasis on understanding biological systems using the scientific, technical, and conceptual resources of physics.

Many of the achievements of science to date have come from a reductionist strategy, in which scientists attempt to decompose the object under study into ever simpler components and to understand those components with ever increasing quantitative precision. Physics has been particularly successful in this regard. In contrast, much of biology has traditionally been less quantitative and more descriptive in character, both because biological systems are so complex and because some of the key explanatory concepts lie at a more abstract level. Examples of such complex phenomena include coding and processing of genetic information by DNA, and information representation, coding, and processing by the nervous system. However, recent advances in biophysical measurement and in molecular biology are beginning to allow detailed physical understanding of biological phenomena that were previously understood only in qualitative terms.[1] P-21 is well placed by virtue of its capabilities and research interests to contribute significantly to this important trend in the biosciences.

In addition to the goal of achieving a physical understanding of biological phenomena, research in P-21 shares a number of other common characteristics. Specifically,

- we investigate the relationships between structure, dynamics, and function of biological phenomena over a wide range of scales (*e.g.*, from biomolecules through the human brain);
- we make extensive use of detection, imaging, and reconstruction techniques (*e.g.*, x-ray crystallography, single molecule electrophoresis, magnetic resonance imaging [MRI], and magnetic field measurements using technologies based on superconducting quantum interference devices [SQUIDs] as shown in Fig. 1);
- we attempt to achieve a detailed interplay between high-resolution physical measurement and large-scale computational modeling and analysis of complex systems;

- we develop new facilities in support of our scientific and technical goals, including a dedicated x-ray beam line for protein crystallography at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory, a large-bore MRI facility, a high-speed electronics laboratory and fabrication facility, and a growing SQUID applications laboratory at Los Alamos;
- we depend heavily on the tight connection and daily interplay between biologists and physical scientists within the group, the division, and the Laboratory; and
- we apply the knowledge, techniques, and capabilities developed in our biological studies to problems of national security and those of specific interest to the Laboratory when our ongoing efforts can offer unique solutions and significant mutual benefit.

During the past two years, P-21 had a number of major accomplishments, including the addition and rapid integration of the world-class high-speed electronics team previously in the Hydrodynamics and X-Ray Physics Group (P-22), winning two of the Laboratory's total of four R&D 100 Awards for 1998, significant contributions to the formation of a new $60M National Foundation for Functional Brain Imaging, and helping to formulate and launch a new national research initiative in structural genomics. Our scientific and technical activity lies in six major areas, which are discussed individually below.

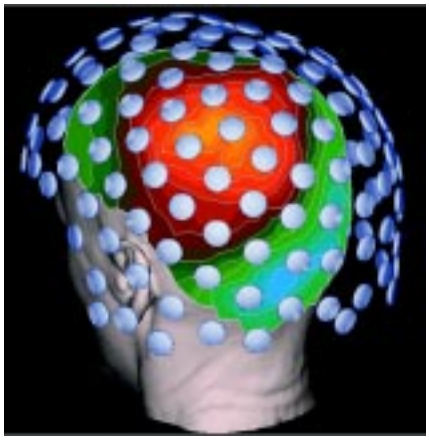


Fig. 1 Our whole-head MEG system uses SQUID sensors to record the magnetic fields produced by active populations of neurons.

Protein Structure, Dynamics, and Function

Our studies of protein dynamics aim to describe protein motion in atomic detail and understand the consequences of protein dynamics for protein function. We have extended our original work on kinetic x-ray crystallography of myoglobin[2] to the understanding of proteins important for bioremediation of trichloroethylene (TCE) and other soil and groundwater pollutants. P-21 is part of a multidisciplinary Los Alamos effort that seeks to enable bioremediation of TCE by genetically engineered microorganisms. The first step in this effort is obtaining a thorough understanding of the enzymatic mechanisms by which TCE can be degraded. In collaboration with scientists at universities across the country, as well as at the Max Planck Institute in Germany, P-21 scientists have begun to unravel the mystery surrounding the mechanism of one class of enzymes that might be engineered to degrade TCE: the cytochrome P-450s. P-450s bind molecular oxygen, split the dioxygen bond, and insert one oxygen atom into organic substrates. This can be the first step in the biodegradation of TCE. The reaction is also a crucial step in steroid hormone synthesis, and P450s are likely to be important in developing drugs to treat breast and other cancers.

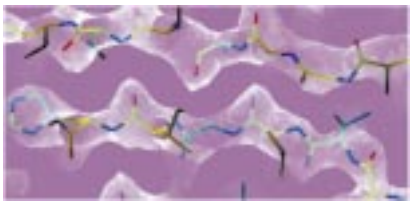


Fig. 2 An electron density contour map (pink) of an atomic model of that density (colored sticks). Solutions for protein structures such as this will be the primary emphasis of the protein crystallography program at the X8-C beamline.

To support our rapidly increasing efforts in protein crystallography, P-21 has converted beamline X8-C at the Brookhaven's NSLS to dedicated use for x-ray crystallography of proteins. Built originally for the Physics Division's weapons physics applications, the transition plan for this beamline was initiated in 1996 and completed in early 1998. To support this dedicated facility, we established an NSLS Participating Research Team consisting of the Los Alamos Integrated Structural Biology Resource, the National Research Council of Canada Biotechnology Research Institute, the Department of Energy (DOE) Molecular Biology Institute at the University of California at Los Angeles, the Brookhaven Biology Department, and the Pharmaceutical Division of Hoffman-La Roche, Inc. The primary emphasis at X8-C will be on solving novel protein structures through use of multiwavelength anomalous dispersion (MAD) on small crystals (100 μm and less) under cryogenic conditions (Fig. 2). X8-C is well-suited for this type of experiment because it has optics that deliver a high flux with low bandwidth when compared with other protein-crystallography beam lines at the NSLS.

Over the last year, the structural genome initiative (a systematic approach to obtaining the structures of large numbers of biologically and medically important proteins) has lead to the beginnings of a large-scale national effort with support from the DOE Office of Biological and Environmental Research and the National Institute of Health (NIH). Our goal is to maintain leadership in this area, which we expect to be both an important scientific issue in structural biology and a critical component of future biotechnology (there is already significant interest by major pharmaceutical companies). We were co-organizers of a national workshop entitled "Structural Genomics" which was held at Argonne National Laboratory in January 1998. The meeting was attended by internationally prominent figures in structural biology and genomics, as well as by representatives from the offices of DOE, NIH, the National Science Foundation, and other sponsors. The workshop produced an enthusiastic consensus that structural genomics will be an important part of the post-genome biological landscape. The meeting was widely reported in the scientific press.[3,4] In collaboration with members of the Life Sciences Division and the UCLA Molecular Biology Institute, we have begun a pilot project in structural genomics including large-scale overexpression, purification, and crystallization of proteins from a thermophilic bacterium. This project is expected to produce approximately 60 novel structures over the next three years, almost all of which will be solved by MAD techniques on beamline X8-C.

Functional Brain Imaging

A recent unpublished NIH position paper states "Brain imaging is one of the most rapidly advancing fields in science today. More than any other area of biology, it is a field in which the progress of research is dependent on improving technologies and computational

power. . . [R]apid improvements in brain imaging methods provide our best hope for understanding brain mechanisms that play a role in mental illness and, eventually, for improving our ability to diagnose, treat, and prevent neurologically based brain disorders." P-21's effort in functional brain imaging focuses on the combined use of magnetoencephalography (MEG), anatomical MRI, functional magnetic resonance imaging (fMRI), and optical imaging techniques to develop improved techniques for noninvasive imaging of the human brain. High-resolution MEG arrays and optical imaging techniques are also used to image neural activity directly from the brains of experimental animals (Fig. 3). Together with collaborators at the University of New Mexico School of Medicine, Albuquerque Regional Federal Medical Center in New Mexico, Massachusetts General Hospital in Boston, and the University of Minnesota School of Medicine in Minneapolis, P-21's work in functional brain imaging contributed significantly to the recent formation of the $60M National Foundation for Functional Brain Imaging to be headquartered in Albuquerque.

Members of P-21 are engaged in projects to design improved multichannel magnetic sensors, develop more accurate mathematical models for localizing the electrical and magnetic signals from the brain, validate MEG using known current sources in computational and physical models of the brain, and use MEG to address important questions in basic neuroscience and in research on neurological and psychiatric disorders.

Combining MEG and anatomical MRI with other functional imaging techniques such as fMRI and positron emission tomography (PET) offers the opportunity of increasing the combined spatial and temporal resolution of functional imaging techniques well beyond that of any single method, as noted in the

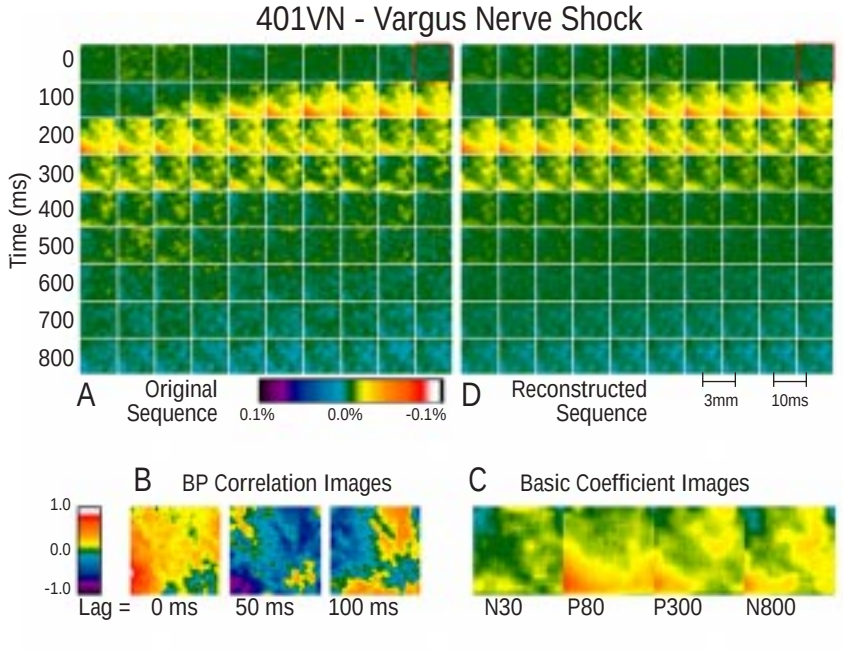


Fig. 3 High-resolution optical imaging techniques allow for images such as this, which accurately measure neural activity directly from the brain.

NIH quotation above. We are engaged in developing mathematical models for combining these alternative forms of brain imaging. This work is part of a nationwide effort to develop three-dimensional (3-D) computational models of the brain in which a variety of structural and functional information can be represented for storage, retrieval, and analysis.

SQUID-Based Sensors and Applications

The goals of our MEG SQUID sensor projects are to develop, test, and evaluate sensor systems, numerical techniques, and computational models for functional imaging of the human brain using MEG. MEG involves the use of SQUIDs to measure magnetic fields associated with human-brain activity. Measurement of the magnetic fields of the brain (which are approximately a billion times smaller than Earth's) requires sensitive magnetic sensors, magnetic shielding from the environment (currently implemented through a shielded room), and advanced signal-enhancement and modeling techniques. Because magnetic fields readily penetrate the skull, MEG offers the potential for noninvasive measurement of brain function in much the same way that computed tomography and MRI allow the noninvasive detection of brain structure. MEG has therefore generated considerable interest in its possible use as a tool in basic neuroscience for functional mapping of the human brain, as a clinical tool for the assessment of neurological and psychiatric disorders, as a possible source of signals for use in the development of neural prosthetics and human-machine interfaces, and in other applied contexts.

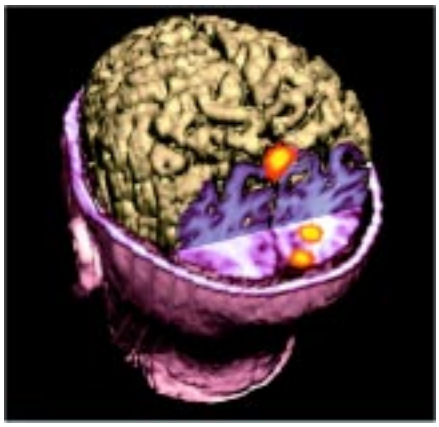
MEG directly measures a physical effect of neuronal currents with temporal resolution not limited by the sluggish vascular response, unlike PET and fMRI that measure hemodynamic changes associated with neuronal activity. High temporal resolution is particularly important for studying neurological disorders such as epilepsy, where temporal information is a major diagnostic, and for fundamental studies of synchronization and oscillatory brain activity. Our whole-head MEG system is based on the P-21 patented principle of superconducting image-surface gradiometry where magnetic sources are imaged on the surface and magnetometers near the surface sense the combined fields as if the sensors were gradiometers (Fig. 4). Fabrication and assembly of this system are nearly complete. This system will play a major role in the National Foundation for Functional Brain Imaging.

Significant progress has also been made in development of novel, improved approaches to the MEG forward and inverse problems. In the case of the forward problem, the two major existing approaches are spherical (or spherical-shell) models and boundary-element models. Spherical models have the advantage of computational simplicity but they can result in significant inaccuracies in regions of the head that depart from spherical geometry. In contrast, boundary-element models are more accurate, but at a significant

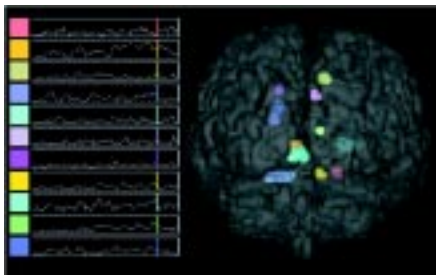
increase in computational complexity. Working with our collaborators, we have developed an alternative to the spherical and boundary-element approaches to the forward problem, termed the weighted multi-sphere approach because it uses multiple spheres fit to the local curvature of the skull. This approach can achieve accuracies approaching those of the boundary-element model with computation time comparable to that of the simple spherical model. With respect to the inverse problem, members of P-21 recently demonstrated a new probabilistic approach based on Bayesian inference, which is described in detail in a research highlight in Chapter 2 of this report. Unlike all other approaches to the inverse problem, this approach does not result in a single "best" solution to the problem. Rather, it estimates a probability distribution of solutions upon which all subsequent inferences are based. This distribution provides a means of identifying and estimating the features of current sources from surface measurements that are most probable among the multiple solutions and can account for any set of surface MEG measurements. The promise of this approach has been demonstrated using computer simulations and experimental data. In particular, we have demonstrated for the first time that information can be extracted not only about the locations of regions of activity but also their extent.

In addition to applications of SQUIDs to MEG and related biological applications, members of P-21 have made significant accomplishments in applying these same sensors to the nondestructive evaluation of nuclear weapons components and materials. As described in detail in a research highlight in Chapter 2 of this report, a SQUID microscope has been designed, built, and tested for applications in the Enhanced Surveillance Program. This system uses a SQUID cooled by liquid nitrogen to map magnetic fields produced by eddy currents in a sample at room temperature. Material defects in the sample (due, for example, to cracks, seams, stress fractures, corrosion, or separation of layers) perturb eddy currents and produce magnetic field anomalies when compared to uniform, defect-free materials. Such anomalies can be detected even if the material defects are located below the surface in deeper layers of the sample. This latter capability is particularly important for nondestructive evaluation of weapons components and materials.

In the first full year of the project, the SQUID microscope team designed, fabricated, and performed successful initial tests of a SQUID microscope based on high-critical-temperature SQUID sensors. This work won P-21's SQUID Microscope Team a Los Alamos Distinguished Performance Award for 1998. Their success was based in part on their ability to exploit P-21's extensive experience in applications of SQUID sensors for noninvasive measurement of human brain function. Given this successful proof of concept, the team is now refining the SQUID microscope design to improve its sensitivity and resolution, to permit operation in magnetically noisy environments, and to use higher-frequency induction fields.



a)



b)

Fig. 4 In our whole head MEG system (shown in Fig. 1), the locations and time courses of active neural populations are calculated using computer models (a) and displayed on MRI images of brain anatomy (b).

Biologically Inspired Hardware, Computation, and Robotics

P-21 is currently making a significant effort in the study of adaptive systems focused on the development of autonomous or semi-autonomous machines, and there is an opportunity for this effort to become much larger. One focus of this work is the performance of simple mobile machines designed primarily to survive in their intended environment. Such devices are controlled by unique analog neural circuits, are often solar powered, and are capable of surprisingly complex behavior, and they may achieve enhanced utility through collective behavior. A second focus concerns the development of more capable robot legs modeled on the legs of insects. The aim is to develop legs that closely integrate the materials, sensors, actuators, power systems, and control structures as much as possible in the way that these components are integrated in the structure of animals. This work is the first step in the development of complex, agile walking robots. It is multi-disciplinary by nature, requiring the participation of materials scientists (for structure, energy systems, and actuators), mechanical and electrical engineers, bioscientists, physicists, and others. Opportunities exist for P-21 to become a major Laboratory and national resource in adaptive hardware, computation, and robotics for national security missions of the DOE Office of Nonproliferation and National Security, the intelligence community, and the Department of Defense (DOD) Advanced Research Projects Agency (DARPA). An expanded effort in this direction would exploit our existing strengths in robotics, engineering, neuroscience, and computation, and would significantly expand our contribution to core Laboratory missions with first-rate science and technology.

A major focus of such an expanded effort will be adaptive and biologically-inspired computation. Millions of years of evolution have endowed organisms with the ability to solve problems that overwhelm even the largest of today's DOE Defense Program stockpile stewardship computers. Biological solutions to these seemingly intractable computational problems involve massively parallel, richly interconnected networks of neurons whose collective activity is essential to their function. Artificial neural networks (ANNs), cellular automata, and other approaches to adaptive computation have achieved considerable notoriety as potential solutions to difficult computational problems because of their similarities to biological neural networks and because, unlike conventional numerical simulations, they do not require a detailed algorithmic solution to the problem a priori. Instead, because of their inherent plasticity and their ability to "learn" from experience, ANNs can be "trained" to solve problems in ways that are not explicit in their initial architecture.

ANNs are ultimately limited, however, because of their inherently rudimentary representation of the computational capabilities of real neurons. In actual neurons, tree-like dendritic structures are the site of a complex analog computation involving

the timing of input spikes, the rapid interplay of voltage- and ion-specific membrane channels, and the active feedback of signals from the cell body back into the dendritic tree.[5] The size of ANNs to date is generally very small on the biological scale, where even the simplest of organisms have networks of hundreds of neurons, and most have vastly more. We believe that it is possible and desirable to begin to design neural networks that are more closely linked to biological neural systems, to use them to address hitherto intractable computational problems that biology appears to have solved in an elegant fashion, and ultimately to use systems of such realistic neurons to build useful devices.

Single Molecule Spectroscopy and Electrophoresis

P-21 and its collaborators have extended their work on the detection and characterization of single molecules in a liquid. The goal of this research is to measure and characterize the spectroscopic properties of individual molecules (Fig. 5). Such spectroscopic measurements can be used to identify the presence of a particular molecular species in an extremely dilute solution, or they can be used to probe the local environment that surrounds an individual molecule. The former capability promises a new level of speed and sensitivity for medical diagnostics, whereas the latter capability makes it possible to study properties of biological systems that cannot be measured when a lack of sensitivity confines measurements to the determination of the average properties of a large ensemble of microenvironments. Thus far, the spectroscopic properties measured at the single-molecule level include emission spectra, fluorescence lifetime, and total emission intensity. Recently the single-molecule spectroscopic approach has been extended to include single-molecule electrophoresis and approaches to ultrasensitive detection of viral and bacterial pathogens in soil and water samples. We are exploring additional applications for basic research and for medical diagnostics.

High-Speed Electronics Team

Already a diverse group, P-21 became more diverse and significantly stronger with the addition in December 1997 of the electronics team formerly in P-22. Previously a key element of the nuclear test program at the Nevada Test Site (NTS), the electronics team refocused its efforts to other defense and civilian needs with the cessation of nuclear testing. We now, quite literally, have the capability within P-21 to take an idea from the "gleam-in-the-eye" stage, through basic and applied research, to a fully developed, fieldable instrument for direct use by sponsors or industrial partners. The electronics team brings substantial capabilities in electronics design, fabrication, and implementation to P-21 that are of great value in their own right and have significant potential for the enhancement of our biological programs. In less than one year, the electronics team has made contributions in all of the focus areas listed above, including exploration of detectors derived from remote

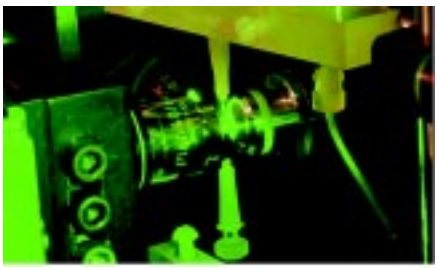


Fig. 5 The single-molecule electrophoretic analyzer detects single labeled molecules in solution.

ultra-low light imaging (RULLI) techniques (see the research highlight on this topic in Chapter 2) for applications in biomedical imaging and single molecule detection, contributions to high-throughput protein purification for the structural genome project, and other areas.

In collaboration with the Life Sciences Cytometry Group (LS-5), the P-21 Electronics Team played a key role in the development of a flow cytometer to be used as part of a suite of instruments by the U.S. Army Chemical and Biological Defense Command. The instrument provides point detection of a biological warfare attack at a forward battlefield location, and rapid identification of the biological warfare agent. The requirements called for the instrument to be exceptionally compact, rugged, and easy to use while precisely identifying a host of bio-agents. The miniature flow cytometer (MiniFCM) successfully completed field tests in September 1998, and 14 instruments are currently in use at Fort Polk, Louisiana.

The electronics team significantly increased the dynamic range of the instrument while also reducing the size of the required electronics (Fig. 6). A unique scheme of data acquisition, which used two ADC's and overlapped their coverage, was used to increase the dynamic range to 16 bits with very low noise. Simultaneously detecting particles in very low channels and high channels was fundamental to the use of the instrument (Fig. 7). In addition, the size of the MiniFCM was reduced by designing acquisition electronics around a multichip module hybrid, which placed 32 integrated circuits on a 30×55-mm substrate. The team also simplified the controls and user interface of the instrument, transforming a clinical instrument controlled by a separate PC and software into a field instrument controlled by five buttons.

Fig. 6 The miniFCM can detect specific bio-aerosols, cells, and spores within 120 seconds. It is self-contained and easily portable, weighing only 60 lbs and measuring 2.5 ft³.

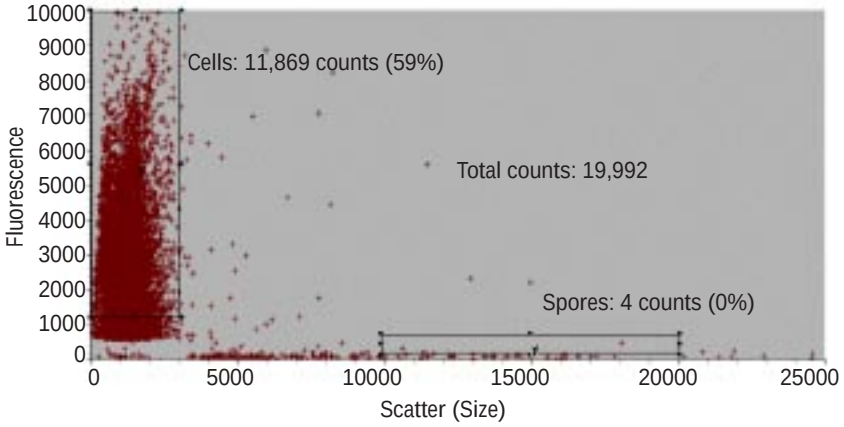


Fig. 7 Bivariate dot plot of size (scatter) versus DNA fluorescence for particles detected during a field trial of the miniFCM at Dugway Proving Ground.* Erwinia herbicola*, a vegetative cell that causes black spots on pears, was released outdoors. The miniFCM was able to detect both the cells (upper left) and spores (lower right) of this pathogen simultaneously, even though the spores are larger and less fluorescent.

The instrument is part of a mobile bio-agent laboratory. It consists of a precisely aligned and focused optical platform, a fluidic system for sample delivery and system decontamination, and a virtual memory extension (VME)-based data acquisition and control system. Unlike commercial flow cytometers, these systems had to be made physically rugged to survive transportation and be immediately on-line for bio-agent detection. This required extremely stabile optical and fluidic components and special detail in every aspect of instrument construction. The result is a remarkably high level of adjustment-free operation, especially when compared to standard commercial instruments.

Further Information

For further information on all of P-21's projects, refer to the project descriptions in Chapter 3 of this progress report. Some of our major achievements are also covered as research highlights in Chapter 2, as mentioned above. These include SQUID microscope development, research on Bayesian methods for addressing the MEG inverse problem, development of RULLI techniques, and the structural genome project.

References

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